

# ISACHEPM

## U.S. Army Center for Health Promotion and Preventive Medicine



TOXICOLOGICAL STUDY NO. 87-3012A-97  
STUDIES ON THE STABILITY OF THE BINDING  
OF TNT RESIDUES TO ORGANIC FRACTIONS  
OF SOIL COMPOST DURING COMPOSTING  
OF TNT-CONTAMINATED SOIL  
OCTOBER 1994 - OCTOBER 1995

Approved for public release; distribution unlimited.

19970508 064

*Readiness Thru Health*

DESTRUCTION NOTICE - Destroy by any method that will  
prevent disclosure of contents or reconstruction of the document

## U.S. ARMY CENTER FOR HEALTH PROMOTION AND PREVENTIVE MEDICINE

The U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) lineage can be traced back over a half century to the Army Industrial Hygiene Laboratory which was established at the beginning of World War II under the direct jurisdiction of The Army Surgeon General. It was originally located at the Johns Hopkins School of Hygiene and Public Health with a staff of three and an annual budget not to exceed three thousand dollars. Its mission was to conduct occupational health surveys of Army-operated industrial plants, arsenals, and depots. These surveys were aimed at identifying and eliminating occupational health hazards within the Department of Defense's (DOD) industrial production base and proved to be extremely beneficial to the Nation's war effort.

Most recently, the organization has been nationally and internationally known as the U.S. Army Environmental Hygiene Agency (AEHA) and is located on the Edgewood area of Aberdeen Proving Ground, Maryland. Its mission had been expanded to support the worldwide preventive medicine programs of the Army, DOD and other Federal agencies through consultations, supportive services, investigations and training.

On 1 August 1994, the organization was officially redesignated the U.S. Army Center for Health Promotion and Preventive Medicine and is affectionately referred to as the CHPPM. As always, our mission focus is centered upon the Army Imperatives to that we are optimizing soldier effectiveness by minimizing health risk. The CHPPM's mission is to provide worldwide scientific expertise and services in the areas of:

- Clinical and field preventive medicine
- Environmental and occupational health
- Health promotion and wellness
- Epidemiology and disease surveillance
- Related laboratory services

The Center's quest has always been one of customer satisfaction, technical excellence and continuous quality improvement. Our vision is to be a world-class center of excellence for enhancing military readiness by integrating health promotion and preventive medicine into America's Army. To achieve that end, CHPPM holds everfast to its core values which are steeped in our rich heritage:

- Integrity is our foundation
- Excellence is our standard
- Customer satisfaction is our focus
- Our people are our most valuable resource
- Continuous quality improvement is our pathway

Once again, the organization stands on the threshold of even greater challenges and responsibilities. The CHPPM structure has been reengineered to include General Officer leadership in order to support the Army of the future. The professional disciplines represented at the Center have been expanded to include a wide array of medical, scientific, engineering, and administrative support personnel.

As the CHPPM moves into the next century, we are an organization fiercely proud of our history, yet equally excited about the future. The Center is destined to continue its development as a world-class organization with expanded preventive health care services provided to the Army, DOD, other Federal agencies, the Nation, and the world community.

Michael A. Major  
U.S. Army Center for Health Promotion and Preventive Medicine  
Building 568  
Fort Detrick, MD 21702

John C. Amos.  
Geo-Centers, Inc.  
Fort Washington, MD 21702

Winifred G. Palmer  
U.S. Army Center for Health Promotion and Preventive Medicine  
Building 568  
Fort Detrick, MD 21702

# DISCLAIMER

The views, opinions, and/or findings contained in this report are those of the authors and should not be construed as official Department of the Army position, policy, or decision, unless so designated by other official documentation.



REPLY TO  
ATTENTION OF

**DEPARTMENT OF THE ARMY**  
**U.S. ARMY CENTER FOR HEALTH PROMOTION AND PREVENTIVE MEDICINE**  
**5158 BLACKHAWK ROAD**  
**ABERDEEN PROVING GROUND, MARYLAND 21010-5422**

**EXECUTIVE SUMMARY**  
**TOXICOLOGICAL STUDY NO. 87-3012A-97**  
**STUDIES ON THE STABILITY OF THE BINDING**  
**OF TNT RESIDUES TO ORGANIC FRACTIONS**  
**OF SOIL/COMPOST DURING THE COMPOSTING**  
**OF TNT-CONTAMINATED SOIL**  
**OCTOBER 1994 - OCTOBER 1995**

The 2,4,6 isomer of trinitrotoluene (TNT) and its environmental transformation products are the most important munitions-derived pollutants encountered at military installations. Large amounts of these compounds have been released into the environment during manufacturing and demilitarization of ordnance. Remediation of sites contaminated with explosives is required by current statutes, and cleanup criteria have often been set on the order of 25 mg/Kg for TNT. The U.S. Army Environmental Center (formerly the U.S. Army Toxic and Hazardous Materials Agency) has developed composting methods for treatment of TNT-contaminated soils. While composting is effective in converting TNT to an assortment of other compounds, and in binding these to soil, little if any of the TNT is mineralized. It is known that TNT can be transformed in soil environments, either by oxidation of the methyl group or reduction of nitro groups. However, little knowledge exists about the covalent bonds that fix these metabolites to soil or compost.

This report covers testing done at this laboratory to determine the stability of the bonds that fix residues to the soil/compost matrix.

*Readiness thru Health*

## CONTENTS

Paragraph	Page
1. REFERENCES .....	1
2. AUTHORITY .....	1
3. PURPOSE .....	1
4. GENERAL .....	1
a. Introduction .....	1
b. Composting .....	2
c. Cleavage of the Aromatic Ring .....	2
d. Oxidation of the Methyl Carbon .....	4
e. Reduction of the Nitro Group .....	4
f. Binding Contaminants to Organic Fractions in Soil or Compost .....	6
g. TNT-Residues in Compost/Soil Systems .....	9
5. METHODS .....	10
a. Preparation of Compost .....	10
b. Extraction and Hydrolysis Procedures .....	10
c. Analytical Methods .....	11
6. RESULTS and DISCUSSION .....	11
7. CONCLUSIONS .....	12



REPLY TO  
ATTENTION OF

DEPARTMENT OF THE ARMY  
U.S. ARMY CENTER FOR HEALTH PROMOTION AND PREVENTIVE MEDICINE  
5158 BLACKHAWK ROAD  
ABERDEEN PROVING GROUND, MARYLAND 21010-5422

MCHB-DC-THE

TOXICOLOGICAL STUDY NO. 87-3012A-97  
STUDIES ON THE STABILITY OF THE BINDING  
OF TNT RESIDUES TO ORGANIC FRACTIONS  
OF SOIL/COMPOST DURING COMPOSTING  
OF TNT-CONTAMINATED SOIL  
OCTOBER 1994 - OCTOBER 1995

1. REFERENCES. A list of references is in Appendix A.
2. AUTHORITY. Contract No. 73710676-87-3012 states that this work is funded by the U.S. Army Corp of Engineers.
3. PURPOSE. This report covers testing done at this laboratory to determine the stability of the bonds that fix residues to the soil/compost matrix.
4. GENERAL.

a. Introduction.

(1) Composting is currently the only bioremediation method for explosives-contaminated soils that has progressed through the field trial stage. It has been accepted by the state of Oregon and the U.S. Environmental Protection Agency Region X for remediating the explosives-contaminated lagoon sediments at the U.S. Army Umatilla Depot Activity (UMDA) site at Hermiston, Oregon.

(2) In the most successful of the field composting demonstrations, 30 vol percent of contaminated soil was mixed with an amendment mixture consisting of 30 vol percent cow manure, 25.4 vol percent sawdust, 25.4 vol percent alfalfa, 14.3 vol percent chopped potato waste, and 4.9 vol percent chicken manure (Lowe et al., 1993) and was arranged in windrows. The windrows were tested in aerated and nonaerated versions, both with daily turning for a period of 40 days.

(3) Chemical and toxicological tests of the composted soil product demonstrated greater than 98 percent reductions in acetonitrile extractable and leachable explosives and extractable bacterial mutagens and approximately 90 percent reductions in leachable compounds toxic to *Ceriodaphnia dubia* (Griest et al., 1994 and 1995). Greenhouse microcosm tests of plants and invertebrates with the composted soil product showed small inhibitory effects on the germination and development of some plant species and, conversely, initial enhancement of earthworm size and population (Gunderson et al., 1994; Griest et al., 1994). The overall results suggested that plant and animal populations could be reestablished in the land-applied product.

b. Composting.

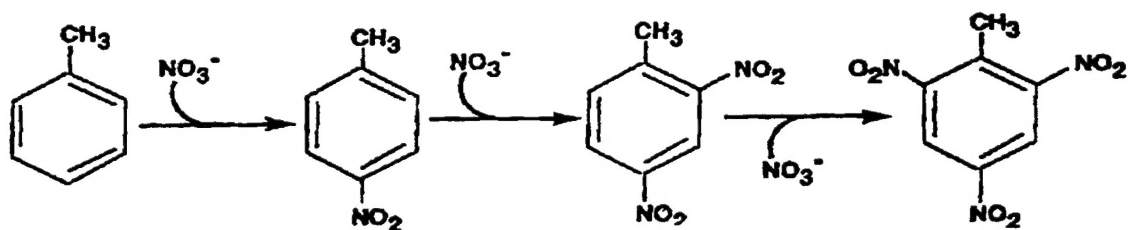
(1) While composting is effective in converting TNT to an assortment of other compounds, little if any of the TNT is mineralized (complete oxidation of the carbon skeleton of organic molecules to CO<sub>2</sub>) (Osmon et al., 1977; Caton et al., 1993). TNT is degraded by natural processes in many environments, but the rates of decay are normally slow and the meta-substituted aromatic ring structure is usually modified, rather than destroyed, by these reactions (Kaplan, 1990; Isbister et al., 1984; Carpenter et al., 1978; Kaplan and Kaplan 1982a; Kaplan and Kaplan, 1982b; McCormick et al., 1976; Walsh, 1990).

(2) TNT can be transformed either by oxidation of the methyl group or reduction of nitro groups (Kaplan 1990; Isbister et al., 1984; Kaplan and Kaplan 1982b; Walsh 1990) (Figure 1). While there may be concern that the suite of compounds generated in this manner may still cause adverse health effects (Funk et al., 1993), current evidence suggests that these compounds are less toxic than TNT (Tan et al., 1992; Wellington and Mitchell, 1991). Further, the toxicity of aqueous leachates and of the compounds extracted into organic solvents, decreases with the duration of composting (Griest et al., 1993; 1995).

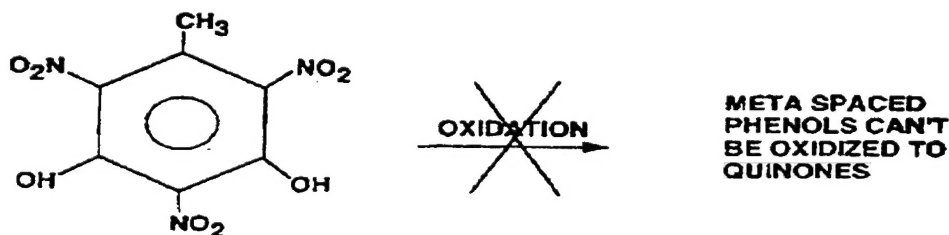
c. Cleavage of the Aromatic Ring.

(1) Biological mineralization of TNT is difficult due to the electrophilic nature and the orientation of the nitro groups. Rieger and Knackmuss (1995) reported that oxygenase reactions are "unknown" for trinitro compounds due to the electron withdrawing power of the three nitro groups. However, we believe that the orientation of the nitro groups is also important. During synthesis of TNT, the initial nitration of the toluene ring occurs preferentially at the ring positions ortho or para to the methyl substituent. Additional nitrations of the ring become more difficult because of the "deactivation" of the ring caused by the cumulative electron-withdrawing effect of each nitro group. An additional result of this deactivation is that new nitro groups are added ortho and para to the methyl group and meta to each other.

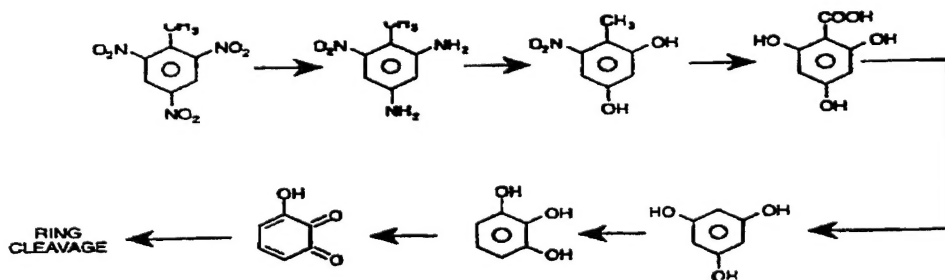




(2) The resultant meta spacing of the three nitro groups ensures that any additional substituents added to the ring will also be emplaced meta to each other. However, biological cleavage of aromatic rings requires emplacement of phenolic substituents oriented ortho or para to each other (Dagley, 1975; Simpson and Evans, 1953). Thus, even if hydroxylations of the aromatic ring were to occur, the required spacing of the phenolic hydroxyl groups could not be achieved without subsequent isomerization. It is likely that the meta orientation of TNT and its resistance to hydroxylation results in the inhibition of biological cleavage and an increase in the persistency of this contaminant in natural environments.



(3) In contrast with the results of field studies, TNT can be biologically mineralized under laboratory conditions. The probable reaction sequence begins with reduction of two or more of the nitro groups to primary amines. In subsequent steps, the amino groups undergo transamination reactions that yield a ring structure with meta substituted phenolic substituents. In later steps, the methyl carbon is removed and rearrangements occur that move the phenolic groups to an ortho configuration. The ring is then oxidatively cleaved (Selivansvskaya et al., 1986).



(4) Although mineralization of the ring carbons of TNT has been demonstrated in the laboratory, it appears that TNT is not utilized as a nutrient for microorganisms in natural environments and during composting. It is probable that the required sequence of reduction, transamination, isomerization, and oxidation is disfavored if other food sources are available.

d. Oxidation of the Methyl Carbon.

(1) The TNT contaminants that reside in aerobic environments at the surface of the soil are often modified by oxidative removal of the methyl carbon to form trinitrobenzene (TNB). Conversion of TNT to TNB is important because the aqueous solubility of TNB is over twice that of TNT (Amos et al., 1997). This property gives the compound a greater potential to leach into groundwater. Demethylation of TNT probably occurs in a multi-step process in which the methyl group is oxidized first to an alcohol, then to an aldehyde, and finally to a carboxylic acid. The carboxylic acid function may then leave the ring as  $\text{CO}_2$ , yielding TNB (Figure 1).

(2) Evidence for the existence of such a pathway was substantiated by Spanggord et al. (1980), who reported the formation of trinitrobenzaldehyde and trinitrobenzoic acid during the degradation of TNT to TNB. It has been reported that oxidation of the methyl group of TNT is mediated by surface catalysis on soil minerals (Checkai et al., 1993a), by ozonation (Kearner et al., 1983), and by the action of sunlight (Spanggord et al., 1980). At sites where the TNT contamination is localized to the soil surface, the concentration of TNB may often exceed that of TNT (Checkai et al., 1993a). Although oxidation of the methyl carbon is prevalent in natural environments, it may be less active during the composting process (Griest et al., 1993). Studies of the formation fate of TNB during composting have, as yet, not been undertaken.

e. Reduction of the Nitro Group.

(1) Reduction of the nitro groups of TNT has pronounced environmental impact because this process alters the aqueous solubility and, consequently, the rate of leaching of these molecules into groundwater. The monoamine reduction products are less soluble than TNT, and the diamino products are more soluble (Amos and Major unpublished). In addition, reduction of the relatively unreactive nitro substituents of TNT produces a suite of substantially more reactive species.

(2) Trinitrotoluene is readily reduced to amino-nitro compounds by natural processes. Reduction of the nitro group that resides para to the methyl group (at the #4 position) is more prevalent than reduction of those at the #2 and #6 positions, as predicted by the work of McCormick et al. (1976). It is known that the 4-amino reduction product is the predominant

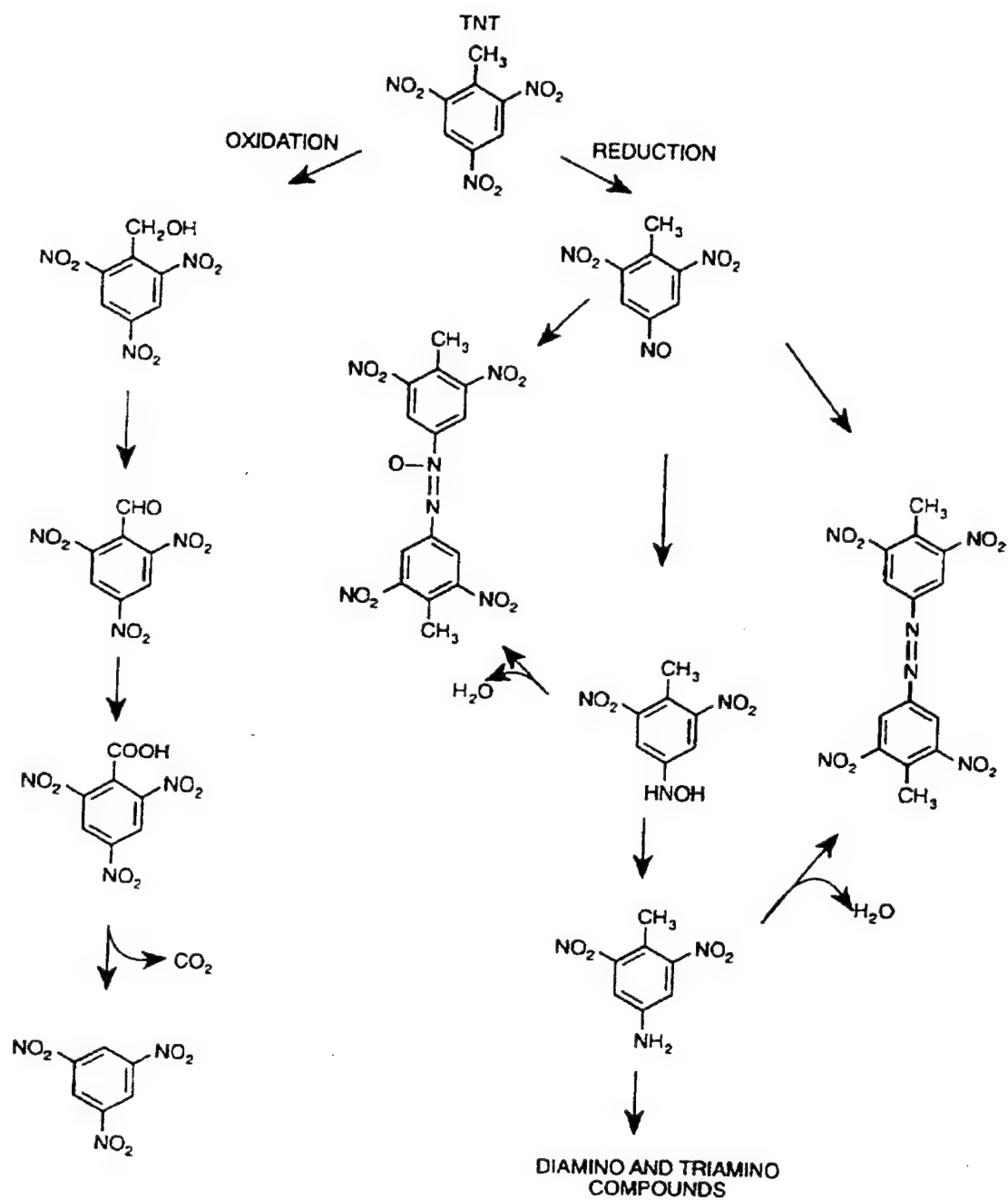


FIGURE 1.

reduced form of TNT in aerobic environments and 2,4-diamino-6-nitrotoluene predominates under mildly acidic (pH 6) anaerobic conditions (Funk et al., 1993). In addition, it has been reported that the 2,4-diamino reduction product can also be generated under aerobic conditions by reaction with certain minerals associated with clays, and that this compound is stable in the surface soil environment (Ainsworth et al., 1993). The triamino reduction product of TNT is not formed at oxygen levels normally encountered in natural soil environments (Kaplan, 1990; Funk et al., 1993).

(3) The reduction of TNT to amino-nitro compounds is a multi-step process involving nitroso and hydroxylamine intermediates (Walsh, 1990) (Figure 1). Hydroxylamines are chemically unstable compounds that can react with nitroso intermediates to form azoxy bonds. This reaction links the two aromatic rings, forming dimers and perhaps higher level polymers (Kaplan 1990; Isbister et al., 1984; McCormick et al., 1976; Walsh 1990; Funk et al., 1993). Hydroxylamines may also undergo disproportionation reactions in which two hydroxylamines react to yield a nitroso and an amine. The latter compounds may then react abiotically to yield azo compounds (Kaplan, 1990; Isbister et al., 1984; Walsh, 1990).

f. Binding Contaminants to Organic Fractions in Soil or Compost.

(1) The reduction of the hydroxylamine to an amine is important from an environmental standpoint because aromatic amines can react with a variety of functional groups to form covalent bonds that link these compounds to the organic fraction of soil or compost (Figure 2). These processes offer the potential for reducing the hazard from TNT-contaminated soils by sequestering transformed products of TNT, as has been suggested for other amino pollutants (Bollag and Myers, 1992).

(2) The covalent binding forces between explosives and composts have not been elucidated, but it is likely that reduction of the nitro substituents must occur prior to generation of covalent linkages. Greene et al. (1985) found that TNT was retained at higher levels in soil columns that were supplemented with glucose. This experiment supports the contention that reduction of nitro groups is needed for binding because the addition of glucose to the leaching mixture would tend to promote anaerobic conditions within the column and, thus, increase the rate of reduction. Evidence that reduction of the nitro groups of TNT is necessary for binding during composting is found in the work of Griest et al. (1994; 1995). These studies show that decreases in extractable TNT in compost were accompanied by increases in acetonitrile extractable TNT-reduction metabolites, during the first few days of composting. Concentration of extractable amino metabolites decreases in later phases of the composting process, as they are bound to substituents of the organic matrix.

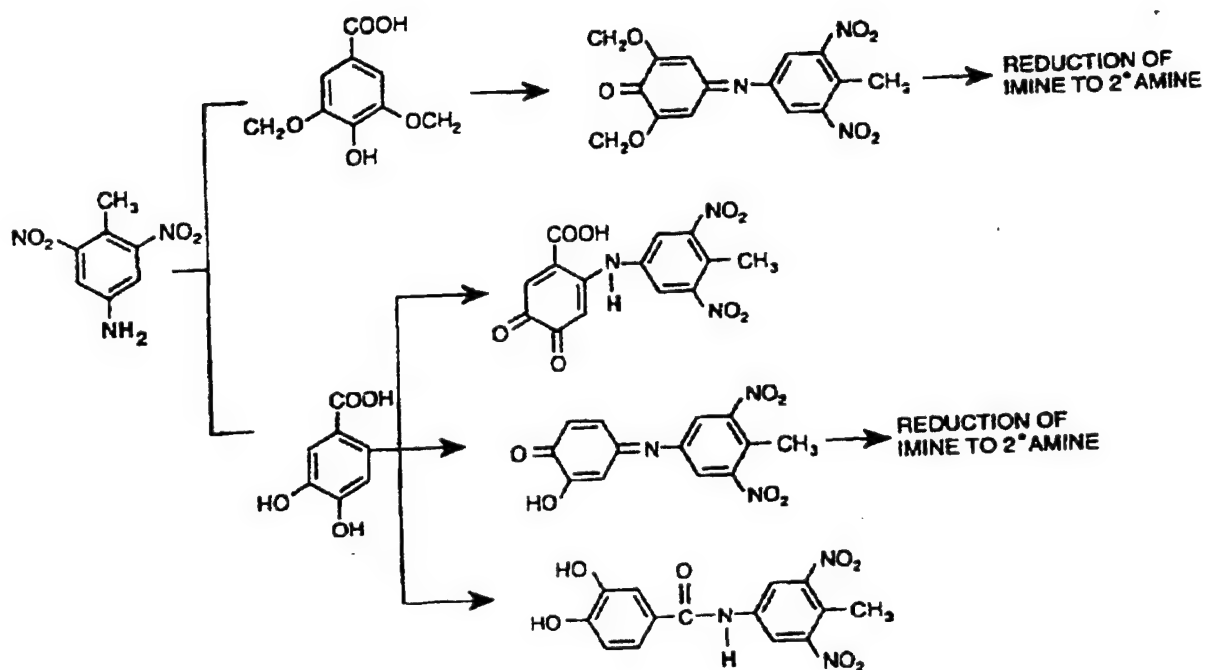


FIGURE 2.

(3) Analogy with the current understanding of the reactivity and incorporation of other aromatic amino pollutants into soil fractions, the binding process for amino reduction products of TNT could involve at least three mechanisms: the formation of imine bonds by reaction of the amino substituents with carbonyl compounds, the formation of secondary amines, and the incorporation of the amino group into a heterocyclic ring via an intermediate secondary amine (Figure 2). The importance of these three mechanisms lies in the relative stabilities of their products. The imine reaction is reversible, leading to the release of the amino product while the amine and heterocyclic structures are expected to be stable in most soil environments. The incorporation of amino compounds by these mechanisms is a result of the processes that generate the humus components of soil (Bollag and Bollag, 1990). During this process, natural multi-functional phenols are polymerized in an oxidative process to form soil humus. Thus, amines are first bound to phenols and are subsequently introduced into the humus complex by the incorporation of the phenol. Evidence for such a pathway is found in the work of Pennington et al., 1995 who demonstrated that the humus fractions represent the largest class of soil organics to which TNT derivatives are bound during composting.

(4) The rate-limiting step in the humification process is probably the conversion of the phenol to a semiquinone radical (a highly reactive free radical generated with some ortho or para substituted phenols) (Schnitzer, 1982). Semiquinone radicals react to form alkoxy linkages with other phenols or phenolic acids during the polymerization reactions of the humification process. It has been reported that phenolic free radicals (perhaps semiquinones) also react with aromatic amines to form imine bonds and thus serve as a means to link these compounds to the developing humus polymer (Bollag and Bollag, 1990).

(5) Imine bonds would probably fail to provide stable linkages between anilines and soil in many environments, because their chemical properties make them susceptible to hydrolysis Parris (1980). A more stable bond would be generated if the imine is subsequently reduced to a secondary amine. A second possible route for in situ formation of secondary amine linkages in soil is described by Parris (1980). In these studies, aromatic amines were reacted with quinones, resulting in the direct formation of a secondary amine linkage between the aniline nitrogen and the ring carbon para to the carbonyl group of the phenol. Evidence that a similar mechanism is involved in linking TNT metabolites to humus has been reported by Major et al. (1995). In these studies, both imine and secondary amino linkages are formed between 4-amino-2,6-dinitrotoluene and polyfunctional phenols in response to catalysis by horseradish peroxidase. In addition, both Parris (1980) and Hsu and Bartha (1976) reported mechanisms by which anilines could be transformed into stable heterocyclic rings. The proposed reaction mechanisms involve formation of a secondary amine linkage between the aniline nitrogen and a carbon of a substituted aromatic ring. In subsequent reactions, an additional bond forms between other functional groups or between the secondary amine nitrogen and a second reactive center yielding the heterocyclic rings.

(6) Formation of a heterocyclic ring by generation of a tertiary amine is a favored reaction for secondary amines because secondary amines do not form imines. Inclusion of one or more of the amino groups that arise from the nitro reduction of TNT into heterocyclic rings, would essentially make the residue an integral part of the soil humus complex. This mechanism is highly desirable for sequestering the transformed TNT, and it should be promoted in the development of optimum composting conditions.

g. TNT-Residues in Compost/Soil Systems.

(1) It follows from the above discussion, that the binding of TNT metabolites to soils may depend in large part on generation of amino functional groups by reduction of nitro substituents. In addition, it is known that anaerobic conditions and low pH favor the rapid formation of amino substituents. Composting, is an oxidative process and is, therefore, not ideal for the reduction of nitro groups. Although reduction of nitro groups occurs under aerobic conditions, the reaction is slower than in anaerobic environments, and the amount of nitroso and hydroxylamine intermediates probably increase (Kaplan 1990; Walsh 1990; Funk et al., 1993). Therefore, increasing the anaerobic character of composts should accelerate production of amino metabolites and, thus, potentiate the binding process. This theory is supported by the work of Lowe et al. (1993) and Griest et al. (1994; 1995), who reported that biotransformation of explosives in field windrow composting was slightly more efficient in nonaerated than in aerated windrows.

(2) An increase in the anaerobic character of a compost system would also tend to decrease the concentration of hydroxylamine intermediates. A significant build-up of hydroxylamines would constitute a serious drawback for composting as a remediation method because composts tend to generate the aerobic, alkaline conditions that best support the condensation of hydroxylamines to azoxy-linked dimers and polymers. Azoxy-linked dimers and higher level polymers are probably bioremediation "dead ends" because they are very poorly soluble in water and are, therefore, theoretically less reactive toward more stable binding processes that occur in solution phase reactions.

(3) Despite the potential for composting to produce azoxy-linked dimers, these compounds were not consistently present in compost systems. Kaplan and Kaplan (1982b) report that azoxy compounds can become the principal form of TNT metabolites under simulated composting conditions. However, some studies that used lower concentrations of TNT in the compost showed little accumulation of these products (Griest et al., 1993). This is consistent with work of Isbister et al. (1984) who reported that TNT modification products were largely absent even with initial TNT concentrations as high as 10,000 ppm. Other studies showed that concentrations of nitro reduction products often arise early in the composting process and decrease with time (Williams et al., 1992; Woodward 1990; Griest et

al., 1995). In the latter studies, azoxy dimers, though not present in organic solvent extracts, were found in aqueous leachates of some composts (Griest et al., 1995). These observations suggests that wet conditions, alkalinity, and the presence of oxygen, enhance the formation of azoxy metabolites. They also suggest that a better understanding of the conditions that support formation of azoxy compounds will enable composting procedures to be developed that minimize formation of these species.

## 5. METHODS.

a. Preparation of Compost. Uniformly ring-labeled  $^{14}\text{C}$ -TNT (98 percent pure) was obtained from NEN/DuPont, Boston, Massachusetts. Twenty mCi  $^{14}\text{C}$ -TNT in 1 mL tetrahydrofuran was added to 2 g soil and air-dried. The  $^{14}\text{C}$ -TNT-labeled soil was mixed with amendments and the compost generated onsite at UMDA by Roy F. Weston, Inc., (West Chester, Pennsylvania). The compost mixture contained 19.8 percent soil, 40.7 percent sawdust/alfalfa hay mixture (50:50), 3.9 percent chicken manure, and 24.2 percent cow manure. This was placed inside a small burlap bag in the middle of an aerated, static compost pile containing TNT-contaminated soil and the same mixture of organic amendments. After 90 d, the  $^{14}\text{C}$ -TNT-labeled compost was allowed to air-dry for 1 week in the dark. The dried compost was coarse ground in a Braun Model 201A coffee grinder for 5 minutes and stored in the dark at 4 °C until needed. The specific activity of the dried compost, 1.6  $\mu\text{Ci}/\text{mg}$ , was determined by oxidation of two samples in a Packard Model 306 Oxidizer followed by counting in a Beckman® model LS5801 liquid scintillation counter.

b. Extraction and Hydrolysis Procedures. Two 100 mg compost samples were extracted with 10 mL of acetonitrile by sonication for 12 hours. The compost and acetonitrile extraction fluid were separated by filtration on Whatman #50 cellulose filter paper. Two 0.5 mL samples of the acetonitrile extraction fluid were mixed with Biodegradable Counting Scintillant (Amersham Inc., Arlington Heights, Illinois) and radioactivity determined by liquid scintillation counting. The extracted compost was allowed to air dry on the filter paper. When dry, the compost and filter paper were placed in a 12 mL reflux apparatus containing 2 mL 12 N HCl, 1 mL water, and 1 mL methanol. The mixture was refluxed for 2 hours and filtered as before. The compost was washed with two 5 mL aliquotes of acetonitrile and the acetonitrile extracts and the hydrolysis solutions pooled, and counted as before. The solids and filter paper were dried and oxidized and radioactivity counted as above.

---

® Beckman is a registered trademark of Beckman Instruments Inc., Fullerton, California.  
The use of trademarks does not mean endorsement by the Army but is intended only to assist in the identification of a product.



c. Analytical Methods.

(1) High pressure liquid chromatography (HPLC) analyses were performed on a Hewlett Packard 1050 HPLC system that consisted of an autoinjector, pumping module, and ultra violet (UV) absorbance detector. A wavelength of 244 nm was used in all studies and signal integration was performed with a Hewlett-Packard 3396A integrator. The HPLC standards were prepared at 1,000 mg L<sup>-1</sup> in acetonitrile and serially diluted for use as controls and calibration standards. Controls were analyzed before and after each batch of samples. The HPLC radioisotope detection was accomplished using a Packard Radiomatic Flo-one Beta Series A-100 detector, with Packard Flo-Scint II reagent, in a volume to volume ratio of 3:1 reagent: mobile phase. HPLC grade solvents were purchased from Baxter (Muskegon, Michigan). An isocratic HPLC method was used for all testing. The mobile phase was a mixture of 69.3 percent water, 29.2 percent methanol, and 1.5 percent tetrahydrofuran. The mixture was pumped at a flow rate of 1.0 mL min<sup>-1</sup> through a 4.6x75 mm LC-8 column of 3 µm particle size (Supelco Inc.).

(2) The table lists the tested compounds used as standards.

TABLE. List of Test Compounds Used as Standards.

TNT	TNB
4-amino-2,6-dinitrotoluene	2-amino-4,6-dinitrotoluene
2,4-dinitrotoluene,	2,6-dinitrotoluene
4,6-diamino-2-nitrotoluene,	2,6-diamino-4-nitrotoluene,
4,4'-azoxy-2,6-dinitrotoluene,	2,2'-azoxy-4,6-dinitrotoluene,
2,6-dinitro-para-cresol,	4,6-dinitro-ortho-cresol,
2,4,6-trinitrophenol,	2,4,6-trinitrobenzaldehyde.

6. RESULTS AND DISCUSSION.

a. Current research demonstrates that a variety of bonds may link TNT residues to composts. Although some of these associations are quite strong, others are known to be susceptible to hydrolytic processes (Williams et al., 1992; Caton et al., 1993) and may, therefore, lack sufficient stability to resist degradation by environmental forces. Studies of <sup>14</sup>C-TNT-inoculated compost at Oak Ridge showed that 98.8 percent of the <sup>14</sup>C-activity becomes bound to compost by the end of the composting cycle. Caton et al. (1993) reported that the majority of the <sup>14</sup>C-labeled material is bound to the soil by bonds that resist extraction

with organic solvent and are only slowly broken by alkaline hydrolysis. More stable linkages are also formed, but these are generated much more slowly and involve only a small portion (3 to 5 percent) of the added TNT.

b. Recent studies conducted at our laboratory at the U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) yielded different results. In these studies,  $^{14}\text{C}$ -TNT-inoculated soil was composted. This differs from the studies at Oak Ridge in which  $^{14}\text{C}$ -TNT was added directly to the completed mixture of soil and amendments at the start of the composting process. Adding inoculated soil to the compost is a more realistic application of the radiolabel. In the USACHPPM work, less than 1 percent of the  $^{14}\text{C}$  from composted  $^{14}\text{C}$ -TNT-contaminated soils could be extracted with organic solvents and only 19 to 22 percent could be released by refluxing for 2 hours in a solution of 6 N HCl/methanol. These differences in behavior may result from the variations in the method of  $^{14}\text{C}$ -TNT inoculation as well as from the differences in the chemistry of alkaline and acidic hydrolysis. Acid hydrolysis may be more appropriate for evaluating the stability of these complexes because nitroaromatics are susceptible to attack by strong bases. Therefore, base hydrolysis may not be attacking the linkage between the compost and the TNT residue, but instead, may be attacking the substituents of the TNT metabolite directly. Moreover, most humic fractions are soluble in base and represent the largest class of soil organics to which TNT derivatives are bound during composting (Pennington et al., 1995). Thus, alkaline treatment may remove the  $^{14}\text{C}$  metabolites as a soluble complex with soil organics.

c. It is important to note that acid hydrolysis did not release detectable quantities of amino metabolites of TNT. The radiolabeled compound(s) released by hydrolysis had an HPLC elution time shorter than any "metabolite" of TNT in our inventory. The radiolabeled product(s) released by composting were not identified. It is possible that the short retention times of the radiolabeled product on reversed phase HPLC is due to the high polarity or ionized state of the TNT metabolite. However, it is also possible that the hydrolysis procedure releases a amino metabolite of TNT that remains linked to a polar or ionized molecule from the humus. Because of the extreme complexity of compost, and the resultant difficulty in detecting specific species, the full scope of metabolites of TNT that are produced during composting and the linkages that bind these metabolites to compost are currently unknown.

7. CONCLUSIONS. It is clear from the above discussion, that a more thorough understanding of the chemistry of the composting process is required before its utility as a remediation process for TNT-contaminated soils can be judged. It is important to know the

Toxicological Study No. 87-3012A-97, Oct 94 - Oct 95

entire suite of metabolites generated in these processes and the nature of the polar products released during acid hydrolysis. In addition, new research efforts must be undertaken to determine the stability of the compost-bound pollutants in a variety of natural environments.

MICHAEL S. MAJOR, Ph.D.  
Environmental Chemist  
Health Effects Research Program

## APPENDIX A

### REFERENCES

Amos, J.C., M.A. Major. Laboratory notebook entry 7 December 1995.

Amos, J.C., M.A. Major, R.T. Checkai, and M. Simini, 1997. The effects of humic acid and noionic surfactant on the aqueous solubility of TNT, TNB, RDX, and HMX. U.S. Army Center for Health Promotion and Preventive Medicine. Technical Report No. 87-3012-96.

Ainsworth, C.C., S.D. Harvey, J.E. Szecsody, M.A. Simmons, V.I. Cullinan, T.C. Resch, G.M. Mong, 1993. Relationship between the leachability characteristics of unique energetic compounds and soil properties. Final Report on Project Order No. 91PP1800 U.S. Army Biomedical Research and Development Laboratory, Ft Detrick, Maryland.

Carpenter, D.F., N.G. McCormick, J.H. Cornell, and A.M. Kaplan. 1978. Microbial transformation of <sup>14</sup>C-labeled 2,4,6-trinitrotoluene in an activated-sludge system. *Applied and Environmental Microbiology*. 35: 949-954.

Caton, J.E., C.-h. Ho, R.T. Williams, and W.H. Griest. 1994. Bound fractions of explosives transformed by composting. *Journal of Environmental Science and Health*, A29(4):659-670

Checkai, R.T., M.A. Major, R.O. Nwanguma, J.C. Amos, C.T. Phillips, R.S. Wentzel, and M.C. Sadusky. 1993a. Transport and fate of nitroaromatic and nitramine explosives in soils from open burning open detonation operations at Anniston Army Depot. ERDEC Technical Report No. 135.

Checkai, R.T., M.A. Major, R.O. Nwanguma, J.C. Amos, C.T. Phillips, R.S. Wentzel, and M.C. Sadusky. 1993b. Transport and fate of nitroaromatic and nitramine explosives in soils from open burning open detonation operations at Radford Army Ammunition Plant. ERDEC Technical Report No. 133.

Dagley, S. 1975. Microbial degradation of organic compounds in the biosphere. *American Scientist*. 63: 681-689.

Funk, S.B., D.J. Roberts, D.L. Crawford, and R.L. Crawford. 1993. Initial phase optimization for the bioremediation of munitions-contaminated soils. A Publication of the Idaho Agricultural Experiment Station. Moscow, Idaho.

Greene, B., D.A. Kaplan, and A.M. Kaplan. 1985. Degradation of pink water compounds in soil-TNT, RDX, HMX. U.S. Army Natick Research and Development Center Technical Report No. 85/046. AD-A157954.

Griest, W.H., R.L. Tyndall, A.J. Stewart, J.E. Caton, A.A. Vass, C.-h. Ho, and W.M. Caldwell. 1995. Chemical and Toxicological testing of windrow composts from explosives-contaminated sediments. *Environmental Toxicology and Chemistry*. 14(1):51-59.

Griest, H.R., A.J. Stewart, R.L. Tyndall, J.E. Caton, C.-h. Ho, K.S. Ironside, W.M. Caldwell and E.L. Tan. 1993. Chemical and toxicological testing of composted explosives-contaminated soil. *Environmental Toxicology and Chemistry*. 12(6):1105-1116.

Griest, W.H., A.J. Stewart, C.-h. Ho, R.L. Tyndall, A.A. Vass, J.E. Caton, and W.M. Caldwell. 1994. Characterization of explosives processing waste decomposition due to composting. Final report, ORNL/TM-12812, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

Gunderson, C.A., J.M. Kostuk, M.H. Gibbs, G.E. Napolitano, L.G. Wicker, J.E. Richmond, and J.J. Stewart. 1994. Ecological validation of a new remediation technology: does composting TNT-contaminated soil make it suitable for land application? Proceedings of the 20th Environmental Symposium and Exhibition: Department of Defense Environmental Security-Strategies for the 21st Century. Vol 1, American Defense Preparedness Association, San Antonio, Texas, March 14-17.

Isbister, J.D., G.I. Anspach, J.F. Kitchens, and R.C. Doyle. 1984. Composting for decontamination of soils containing explosives. *Microbiological*. 7: 47-73.

Kaplan, D. L. 1990. Biotransformation pathways of hazardous energetic organonitro compounds, pp 155-182. In D. Kamely, A. Chakrabarty and G.S. Omenn (eds.), *Advances in applied biotechnology*, 4: Biotechnology and Biodegradation, Portfolio Publishing Company, Texas.

Kaplan, D.L., and A.M. Kaplan. 1982a. 2,4,6-Trinitrotoluene-surfactant complexes: Decomposition, mutagenicity and soil leaching studies. *Environmental Science and Technology*. 16: 566-571.

Kaplan, D.L. and A.M. Kaplan. 1982b. Thermophilic biotransformations of 2,4,6-trinitrotoluene under simulated composting conditions. *Applied and Environmental Microbiology*. 44: 757-760.

Kearney, P.C., Q. Zeng, and J.M. Ruth. 1983. Oxidative pretreatment accelerates TNT metabolism in soils. *Chemosphere*. 12: 1583-1597.

Lowe, W.L., T.J. Marks, J.O. Hammel, and L. Myers, 1993. Windrow composting demonstration for explosives-contaminated soils at U.S. Army Umatilla Depot Activity (UMDA), Hermiston Oregon. U.S. Army Report CETHA-75-CR-93043. Roy F. Weston, Inc., West Chester, Pennsylvania.

McCormick, N.G., F.E. Feeherry, and H.S. Levinson. 1976. Microbial transformation of 2,4,6-trinitrotoluene and other nitroaromatic compounds. *Applied and Environmental Microbiology*. 31: 949-958.

Osmon, J.L., C.C. Andrews, A. Tatyrek. 1978. The biodegradation of TNT enhanced soil and compost systems. U.S. Army Armament Research and Development Command Technical Report ARLCD-TR-77032, ADA054375.

Palmer, W.G., J. Beaman, D. Walters, and D.A. Creasia, 1994a. Bioavailability of TNT in composts of TNT-contaminated soil. Abstract, Annual Meeting, March 1994 Abstract. Soc. of Toxicology Annual Conference, *The Toxicologist*, 14(1):1636.

Palmer, W.G., W.G. Reifenrath, J. Beaman, D. Walters, M.A. Major, and D.A. Creasia. 1994b. Skin and lung absorption of TNT residues from composted TNT-contaminated soil. In: *Proceedings of the 18th Annual Army Environmental R&D Symposium*, pp 261-273.

Pennington, J.C., C.D. Hayes, K.F. Myers, M. Ochman, D. Gunnison, D.R. Felt, and E.F. McCormick. 1995. Fate of 2,4,6-trinitrotoluene in a simulated compost system. *Chemosphere*, 30(3):429-438.

Rieger, P-G. and Knackmuss, H-J. 1995. Basic knowledge and perspectives on biodegradation of 2,4,6-trinitrotoluene and related nitroaromatic compounds in contaminated soil. In: *Bioremediation of Nitroaromatic Compounds*. Edited by J.C. Spain. #49 in the series *Environmental Science Research*. Plenum Press, New York, New York.

Selivanovskaya, S.Yu., D.Z. Akhmetovs, and R.P. Naumova. 1986. Terminal steps in preparatory metabolism of 2,4,6-trinitrotoluene in *Pseudomonas fluorescens*. *Mikrobiologia*. 56:6, 1040-1041; as cited in Walsh, reference 11.

Simpson, J.R. and W.C. Evans. 1953. The metabolism of nitrophenols by certain bacteria. *The Biochemical Journal*. 55:24.

Toxicological Study No. 87-3012A-97, Oct 94 - Oct 95

Spanggord, R.J., T. Mill, T. Chou, W.R. Mabey, J.H. Smith, and S. Lee. 1980. Environmental fate studies on certain munitions wastewater constituents lab studies. Menlo Park, California: SRI international. ADA099256.

Tan, E.L., C.-h. Ho, W.H. Griest, and R.L. Tyndall. 1992. Mutagenicity of trinitrotoluene and its metabolites formed during composting. Journal of Toxicology and Environmental Health. 36: 165-175.

Walsh, M.E. 1990. Environmental transformation products of nitroaromatics and nitramines. U.S. Army Corp of Engineers Cold Regions Research & Engineering Laboratory, Special Report 90-2.

Williams, R.T., P.S. Ziegenfuss, and W.E. Sisk. 1992. Composting of explosives and propellant contaminated soils under thermophilic and mesophilic conditions. Journal of Industrial Microbiology. 9: 137-144.

Woodward, R.E. 1990. Evaluation of composting implementation: A literature review. U.S. Army Toxic and Hazardous Materials Agency Report No. TCN 89363. AD-A243 908.